PB81-232035

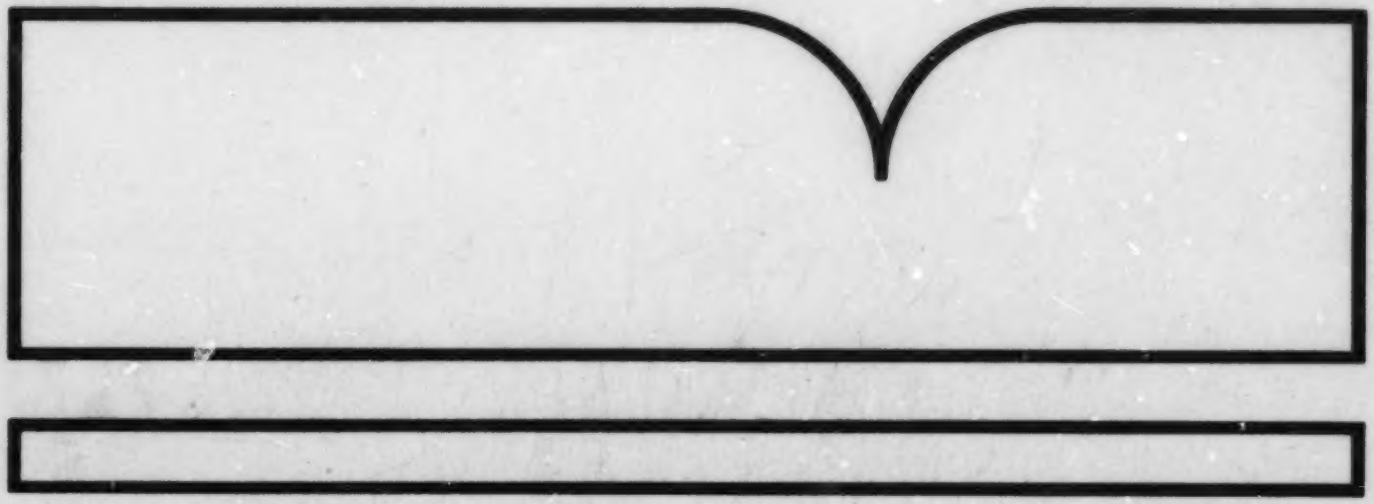
A Scanning Electron Microscope Study of Developing Hamster Tracheal Epethelium in Organ Culture

IIT Research Inst.
Chicago, IL

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

Apr 79



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MS-80-115

1. REPORT NO.	2.	PB81-232035
EPA-600/J-81-029	JOURNAL ARTICLE	

4. TITLE AND SUBTITLE	5. REPORT DATE
A Scanning Electron Microscope STudy of Developing Hamster Tracheal Epithelium in Organ Culture	July 1981
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
L.J. Schiff, M.M. Byrne, K.V. Ketels, W.T. Brown, and J. A. Graham	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Life Sciences Division IIT Research Institute Chicago, IL 60616	C9AN1B
	11. CONTRACT/GRANT NO.
	R-805049-01
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Health Effects Research Laboratory U.S. Environmental Protection Agency Research Triangle Park, N.C. 27711	14. SPONSORING AGENCY CODE
	EPA-600/11

15. SUPPLEMENTARY NOTES

Published In: Differentiation 15: 49-55, 1979

16. ABSTRACT

The ultrastructural surface features of tracheal epithelium at various times of development in organ culture were compared wiht those in the trachea of Syrian golden hamsters of similar age using scanning electron microscopy. Whole tracheal organ cultures, prepared from 3-day-old hamsters, were maintained in HEPES buffered CMRL 1066 medium with 0.2% bovine serum albumin. The ventral epithelial surface of trachea from 3-day-old animals was characterized by numerous microvillous cells, occasional well-developed cilia, and cells containing short cilia representing various stages of ciliary differentiaion. After seven days in culture, an increased number of ciliated cells as well as developing cilia were seen. The epithelium after 14 days in culture appeared to have equal numbers of ciliated and microvillous cells, a pattern strikingly similar to that observed in vivo. After 21 days in culture, groups of well-developed cilia were interspersed with nonciliated cells covered with short, poorly developed surface microvilli. A similar pattern was found in the trachea of comparable age (24 days), with the exception of the microvillous cells; many being dome-shaped containing clusters of microvilli with prominent outlines. The tracheal organ culture, as developed in this investigation, appears to represent an excellent model for studying age-related effects of toxic and infectious agents on ciliated epithelial cells.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	
	20. SECURITY CLASS (This page)	22. PRICE
	UNCLASSIFIED	

EPA Form 2220-1 (9-73)

Differentiation 15, 49–55 (1979)

Differentiation

EPA-J-81-029
Journal Article

A Scanning Electron Microscope Study of Developing Hamster Tracheal Epithelium in Organ Culture*

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The ultrastructural surface features of tracheal epithelium at various times of development in organ culture were compared with those in the trachea of Syrian golden hamsters of similar age using scanning electron microscopy. Whole tracheal organ cultures, prepared from 3-day-old hamsters, were maintained in HEPES buffered CMRL 1066 medium with 0.2% bovine serum albumin. The ventral epithelial surface of trachea from 3-day-old animals was characterized by numerous microvillous cells, occasional well-developed cilia, and cells containing short cilia representing various stages of ciliary differentiation. After seven days in culture, an increased number of ciliated cells as well as developing cilia were seen. The epithelium after 14 days in culture appeared to have equal numbers of ciliated and microvillous cells, a pattern strikingly similar to that observed in vivo. After 21 days in culture, groups of well-developed cilia were interspersed with nonciliated cells covered with short, poorly developed surface microvilli. A similar pattern was found in the trachea of comparable age (24 days), with the exception of the microvillous cells; many being dome-shaped containing cluster of microvilli with prominent outlines. The tracheal organ culture, as developed in this investigation, appears to represent an excellent model for studying age-related effects of toxic and infectious agents on ciliated epithelial cells.

Introduction

The tracheobronchial epithelium of the Syrian golden hamster has proven to be a suitable experimental system for studies of infectious processes [1–3], respiratory carcinogenesis [4, 5], toxicity of chemical agents [6–9], and cell physiology [10, 11]. The maintenance of tracheobronchial epithelium in vitro in the differentiated state under conditions that permit biochemical and morphologic examinations is necessary in order to study the mechanisms of both *cell renewal and differentiation*, under normal and pathogenic conditions.

Scanning electron microscopy (SEM) has permitted investigations of topography because of the ability to scan large surface areas and its great depth of focus. As part of a study to examine the effects produced by energy-related effluents on respiratory epithelium, the SEM has been used to determine early alterations in surface morphology in pollutant-treated tissue. In an effort to establish whole hamster tracheal organ cultures as a system for these studies, we found it necessary to determine whether the epithelium of these cultures have similar structural characteristics to the epithelium in vivo. In this study, the ultrastructural surface features of tracheal epithelium at various times of development in organ culture were compared with those in tracheas of Syrian golden hamsters of similar age using SEM.

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Methods

Organ cultures were prepared from the tracheas of 3-day-old Syrian golden hamsters of the Cr : RGH(SYR) strain by previously described methods [12]. Whole tracheas opened longitudinally along

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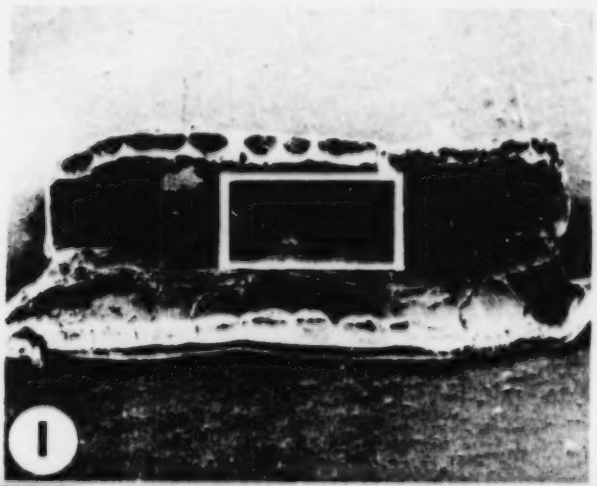
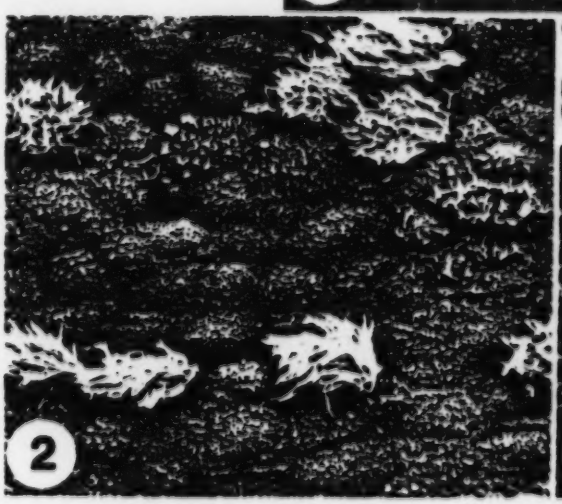
the cartilaginous ventral wall were incubated in groups of three in organ culture dishes (Falcon Plastics, Oxnard, CA). Explants were placed lumen side up on the surface of a silk screen on a stainless steel grid positioned over the center well of the dish containing CMRL 1066 Medium (Gibco, Grand Island, NY) with 0.025 M HEPES buffer, 0.2% bovine serum albumin (fraction V) (Sigma Chemical Co., St. Louis, MO), 250 U penicillin/ml and 250 µg streptomycin/ml. Explants were maintained in an air-tight chamber containing a water-saturated 5% CO_2-air atmosphere at 36° C and the medium and gas were renewed three times a week. Immediately after removal from animals and at 7, 14, and 21 days in culture, six tracheal explants were fixed in Karnovsky's paraformaldehyde-glutaraldehyde phosphate-buffered fixative. The tissues were washed in distilled water, and dehydrated with increasing concentrations of alcohol. Pentyl acetate was then substituted for the alcohol, and the tissues were critical point dried in carbon dioxide. The dried specimens were cemented to a stub, gold coated by vacuum evaporation and examined in a Kent Cambridge Mark II Steroscan SEM at 20 kV.

For in vivo experiments, Syrian golden hamsters were anesthetized with 55 mg/kg sodium methohexital (Brevital sodium, Eli Lilly and Co., Indianapolis, IN) and exsanguinated from the abdominal aorta. The respiratory tract from the larnyx to the bifurcation was excised en bloc, surrounding tissue removed, and opened longitudinally through the cartilaginous ventral wall. Hamsters were killed at 10, 17, and 24 days postnatal, these ages corresponding to the tra-





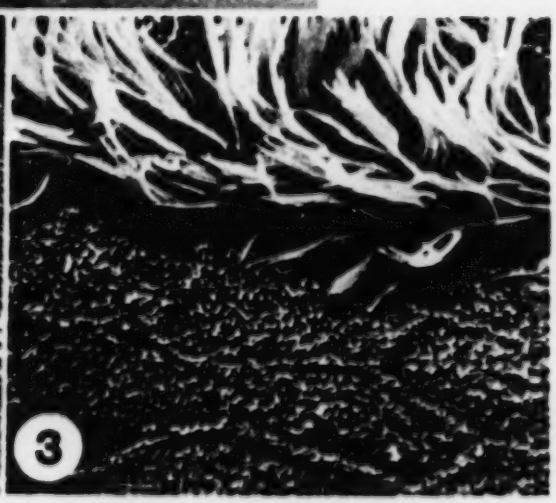


Fig. 1. Trachea from 3-day-old hamster. The ventral surface indicated by the rectangle was used for examination. × 18

Fig. 2. Surface of trachea immediately after removal from 3-day-old hamster showing many nonciliated microvillous cells and occasional ciliated cells. × 1600

Fig. 3. Detail of trachea surface from Fig. 2 showing nonciliated cells covered with dense assembly of microvilli bordered by ciliated cells. × 4000

cheal tissue removed at day 3 and maintained in culture for one to three weeks. Tracheal tissue was fixed and processed as described.

Because regional differences exist in ciliation of tracheal epithelium in hamsters [13], it was necessary to define a specific region for all examinations. The hamster trachea consist of a series of 14 incomplete cartilage rings. The area chosen for examination was the ventral surface of the middle four rings (Fig. 1).

Results

Organ cultures from 3-day-old Syrian golden hamsters were examined at weekly intervals for up to 21 days in culture and compared to tracheas of hamsters of a similar age. The morphology of the ventral surface of the trachea immediately after removal from the animal con-

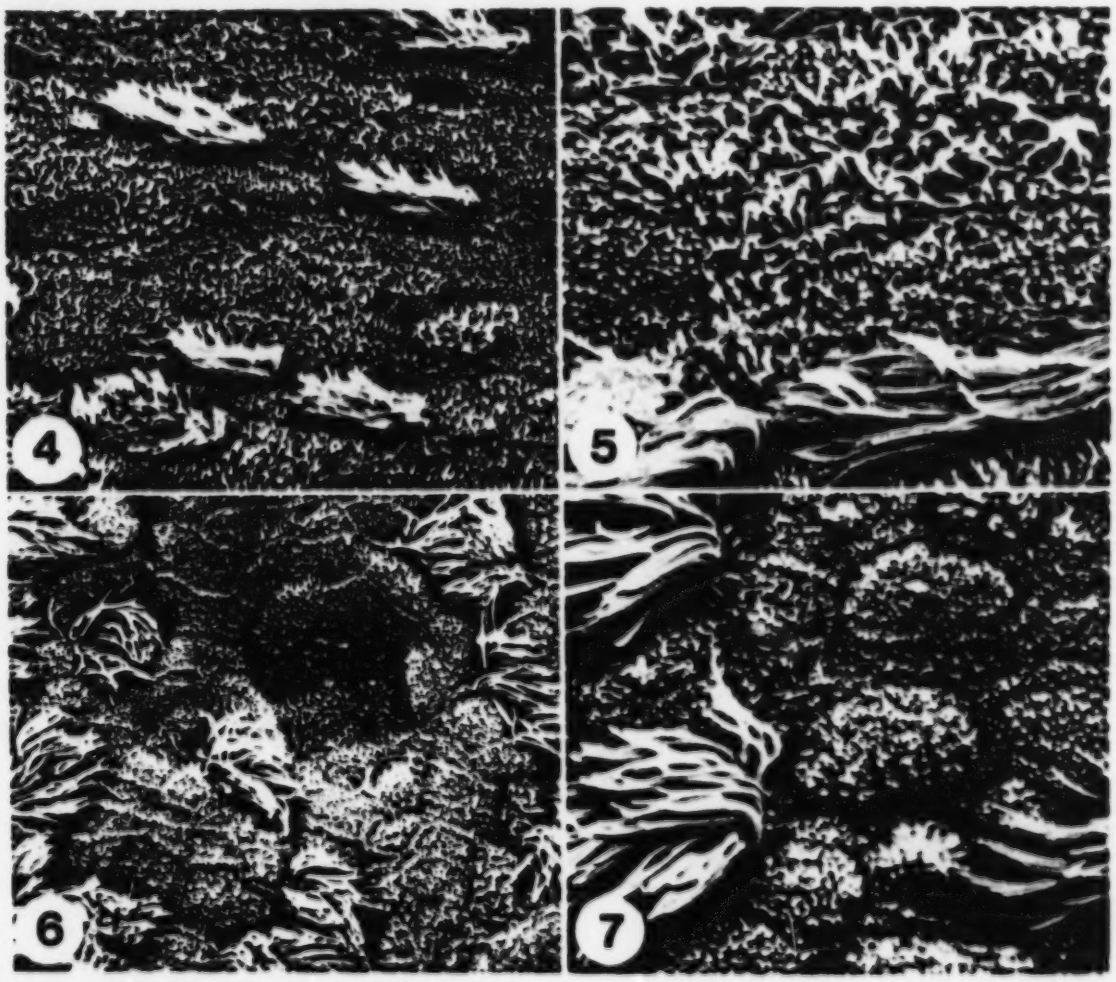


Fig. 4. Surface of trachea after seven days in organ culture showing various stages of ciliary differentiation. × 1600

Fig. 5. Higher magnification of tracheal surface after seven days in culture. The nonciliated cells are covered with many microvilli with neighboring cells showing developing cilia. × 4000

Fig. 6. Tracheal surface from 10-day-old hamster covered with nonciliated microvillous cells with prominent outlines, groups of well developed cilia, and an occasional cell with developing cilia. × 1700

Fig. 7. Detail from Fig. 6 showing polyhedral microvilli-covered cells, lined with tufts of cilia. Note microvilli at the base of ciliated cells. × 4000

sisted of numerous nonciliated cells covered with microvilli and only the occasional presence of well developed cilia (Figs. 2 and 3). Nonciliated cells out-numbered ciliated cells in an approximate 15 to 20 : 1 ratio. In addition to the well developed cilia, a few cells contained short cilia representing various stages of ciliary *differentiation*.

After seven days of culture, an increased number of ciliated cells as well as developing cilia were seen (Fig. 4). As a result of the increased ciliogenesis the epithelial surface acquired an uneven appearance. The length and distribution of the microvilli varied from cell to cell but were shorter than the developing cilia (Fig. 5). The number of nonciliated cells to ciliated cells declined to a ratio

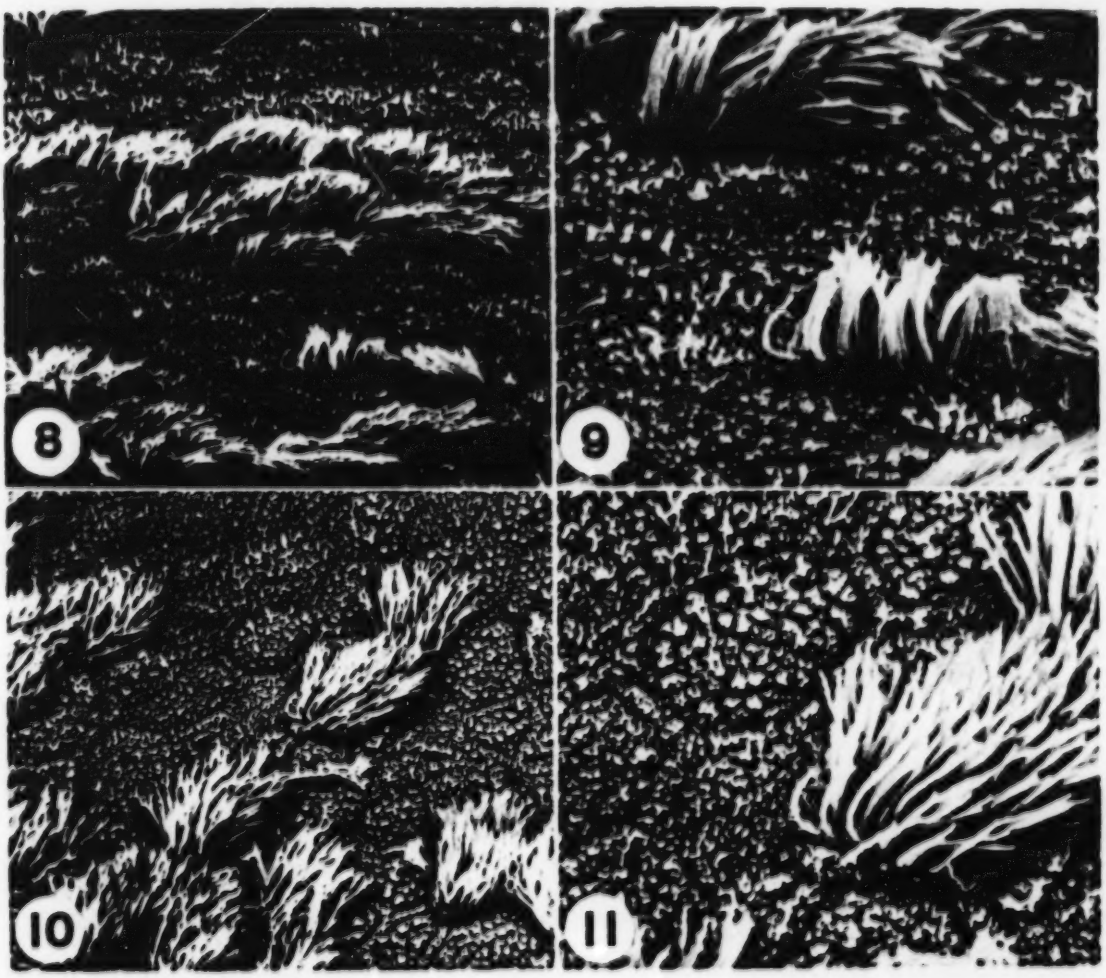


Fig. 8. Tracheal epithelium after 14 days in culture showing surface covered with groups of well-developed cilia randomly dispersed. × 1600

Fig. 9. High magnification from Fig. 8 showing microvillous processes of nonciliated cells arranged in small cluster. × 4000

Fig. 10. Trachea from 17-day-old hamster showing a pattern of surface epithelium similar to tracheas after 14 days in culture. The majority of cells have well defined boundaries. × 1600

Fig. 11. Detail of cell surface from Fig. 10 showing ciliated cells surrounded by nonciliated cells covered with microvilli arranged in clusters. × 4000

of 10 : 1, indicating accelerated ciliogenesis. The density of the ciliary areas in tracheas from 10-day-old hamsters, equivalent to tissue maintained in culture for seven days, was approximately the same (Fig. 6). Groups of ciliated cells were interspersed among microvilli-covered cells which were polyhedral in shape (Fig. 7). In addition to the nonciliated cells predominately covered with microvilli, microvilli were observed at the base of the ciliated cells.

The epithelial surface after 14 days in culture appeared to have equal numbers of ciliated and microvillous cells, a pattern similar to that observed in vivo (17-day-old animals). Well-developed ciliated cells occurred principally in randomly scattered groups, although oc-

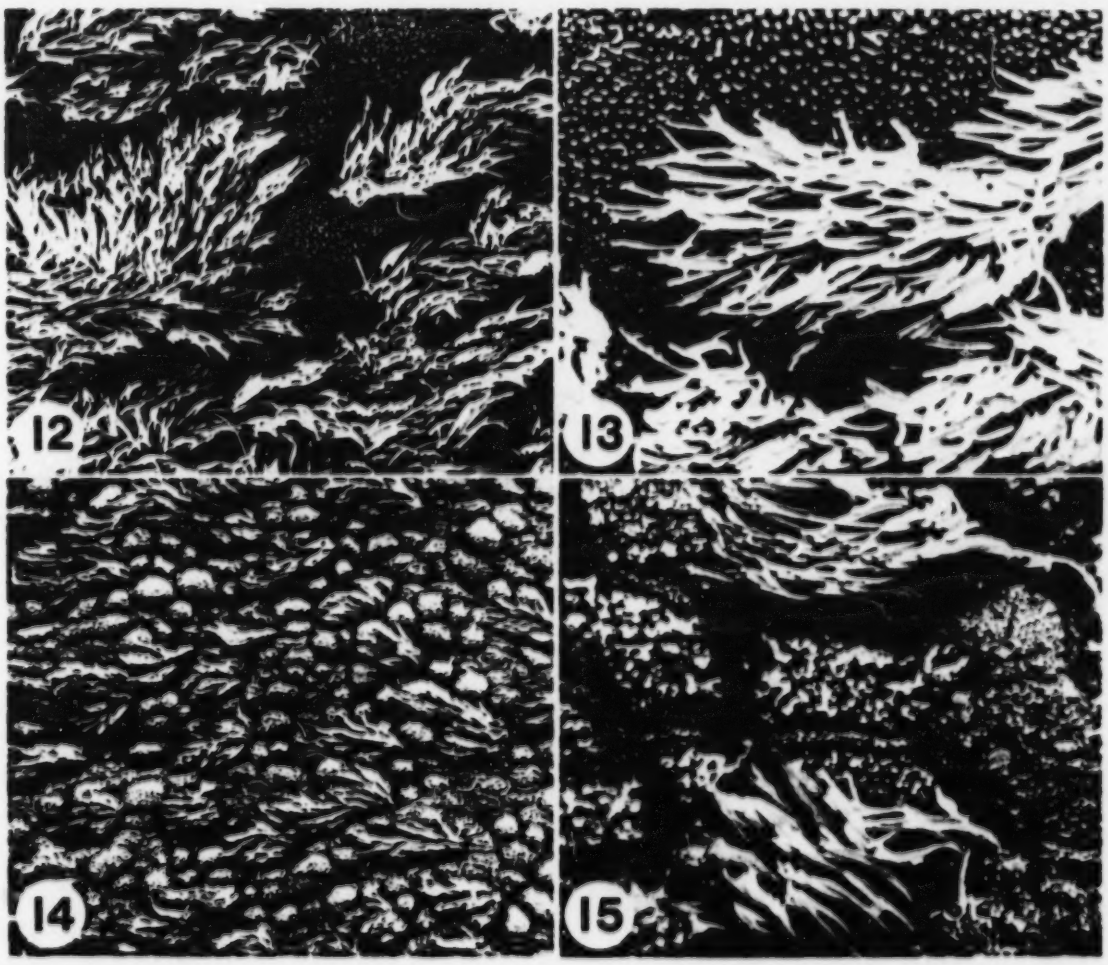


Fig. 12. Surface of trachea 21 days in organ culture showing dense tufts of well-developed cilia and surrounding areas of nonciliated microvillous cells. × 1600

Fig. 13. Detail of surface from Fig. 12 showing cilia and sparsely covered, short microvilli. × 4000

Fig. 14. Tracheal surface of 24-day-old hamster showing numerous nonciliated cells with prominent cell outlines and groups of well-developed cilia. × 800

Fig. 15. High magnification of tracheal surface from 24-day-old hamster showing dome-shaped nonciliated cells with microvilli of various lengths. Ridges of microvilli separate the cellular apices. Occasional droplets of secretory material can be seen. × 4000

casionally isolated tufts from a single cell were seen (Figs. 8 and 10). The microvillous processes of nonciliated cells were arranged in small clusters (Figs. 9 and 11).

After 21 days of culture, the ciliated to nonciliated cell ratio was approximately 1 : 1 (Fig. 12). The same pattern was found in the trachea of hamsters of comparable age (Fig. 14). The cilia were well developed and were arranged in groups interspersed with nonciliated cells. Nonciliated cells of cultured tracheas were sparsely covered with short, poorly developed surface microvilli (Fig. 13). The surface characteristics of nonciliated cells found in the trachea of 24-day-old hamsters can be seen in Fig. 15. Many of the microvillous cells were dome-shaped, with clusters of microvilli on the central elevated area. The apices of these cells were bordered with ridges of microvilli. Small droplets of secretory material were also present.

Discussion

The availability of whole hamster trachea in organ culture suitable for long-term studies offers advantages for the study of age-related effects of toxic and infectious agents on ciliated epithelial cells. To be useful for these studies, however, it is necessary to know whether the tracheal epithelium maintained in vitro has similar structural characteristics as the epithelium in vivo.

Scanning electron microscopy has provided a means of assessing the three dimensional topography of developing hamster tracheal epithelium. The *maturation* of normal tracheal epithelium in vivo and in vitro was associated with distinct ultrastructural surface changes. The topographic characteristics of the tracheal surface were of two predominant cell types; ciliated and nonciliated microvilli-covered. The ventral surface of the trachea from 3-day-old hamsters was characteristically nonciliated cells. After 14 days in culture, equivalent to tracheas of 17-day-old hamsters, an equal number of ciliated cells were interspersed between nonciliated cells. Similar cell types lining the surface of adult mouse [14] and hamster [15] tracheal epithelium have been described. The epithelial surface of rat trachea examined by SEM showed four cell types to be present; ciliated, microvillous, brush, and goblet cells, in that order of frequency [16]. We could not distinguish with certainty between microvillous, brush, and goblet cells.

One of the most striking features of the epithelial surface in uncultured tracheas was the microvilli bordering the polyhedrally shaped nonciliated epithelial cells. In cultured tracheas, the rows of microvilli separating the cell apices could not be distinguished with clarity. Variation in length and distribution of microvilli and scattered small tufts of short cilia randomly-distributed among microvillous nonciliated cells were characteristic of various stages of *ciliary differentiation*. The relationship between microvilli and cilia is unclear. However, in developing hamster tracheal epithelium, ciliogenesis of indifferent cells, i.e., columnar cells showing neither cilia nor mucous granules [15], appears to be independent of mucous differentiation [17]. In epithelium from human bronchus [18] and hamster trachea [15], it appears likely that an intermediate step may exist whereby indifferent cells show both mucous and ciliary differentiation enroute to ciliary differentiation.

An important morphologic characteristic revealed by SEM was the differences in uniformity of the ciliary blanket on the ventral surface of the middle four rings of the trachea. Although equal numbers of ciliated and nonciliated cells exist in 2 to 3-week-old hamsters, a number of large patches of nonciliated cells could be observed. Similar observations were reported in adult hamsters [13], mice [14], and rats [19]. Before changes in surface morphology resulting from the action of air pollutants can be documented, a thorough description of the area in which observations are made must be defined. The necessity to make observations within a specific region of the trachea cannot be over-emphasized.

Acknowledgements: The authors would like to thank Dr. James D. Fenters for valuable comments. This work was supported by Environmental Protection Agency Grant R805049-01.

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Received April 1979/Accepted May 1979